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Membrane trafficking and protein sorting in model systems and specialized primary cells

Membrane trafficking underpins many physiological processes, including secretion, receptor signalling, antigen presentation, and neural networking. Membrane trafficking is also exploited by infectious organisms and toxins to gain entry into the cell. Moreover, many diseases arise from defects in membrane trafficking, including Alzheimer's disease. Our aim is to understand the molecular basis of membrane and protein sorting in the secretory and endocytic pathways in a variety of physiological processes using cultured cells and differentiated primary cells.

a. Membrane trafficking to and from the trans-Golgi network

The trans-Golgi network (TGN) is a major traffic hub. We have identified novel components of the trafficking machinery, including small G proteins and golgins, and have developed a microRNA-based approach to silence these components *in vivo* and explore their physiological function (see Figure 1 as an example). For example, we have demonstrated that the TGN golgins regulate specific transport pathways and now wish to understand how these golgins perform this function and to exploit this information to manipulate trafficking pathways in specialised cells, especially cytokine secretion by immune cells. Major goals of this project are to identify the interactive partners with the TGN golgins and to characterise a recently generated TGN golgin knock-out mouse line. This knowledge will provide new avenues for controlling unwanted inflammation.

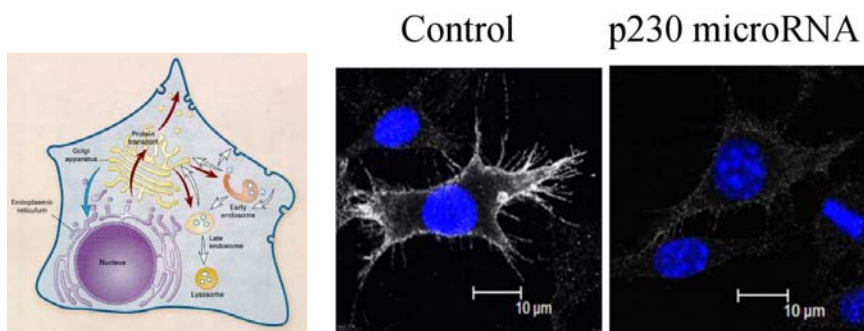


Figure 1. A cartoon of the secretory and endocytic pathways (left-hand side). LPS-activated macrophages showing tumour necrosis factor- α at the cell surface in control cells, whereas the depletion of the TGN golgin, p230, with interference RNA results in a block in Golgi exit of tumour necrosis factor- α and dramatically reduced levels at the cell surface.

b. Signals for endosomal sorting and transport

Many important membrane proteins recycle between the plasma membrane and the Golgi, and that this recycling is essential for their function. A number of membrane transport pathways from endosomes to the TGN have been defined. However, relatively little is known about the targeting signals of cargo proteins responsible for defining their specific retrograde transport routes. Our recent work in the lab has shown that the choice of retrograde pathway *en route* to the TGN requires not only the cytoplasmic domain but is also dependent on information inherent in the transmembrane region. This is a highly novel finding and indicates that a lipid based sorting system contributes to sorting events in the early endosome. This project will now seek to identify the features of the transmembrane domain involved in the sorting process to better understand the endosomal sorting mechanism.

c. Regulated endocytosis

Macropinocytosis is a regulated form of endocytosis and is highly active in macrophages and dendritic cells (antigen presenting cells) where it is a major pathway for the capture of antigens. Despite the importance of this pathway, the molecular basis for the formation and maturation of macropinosomes is poorly defined. This project aims to investigate the role of macropinocytosis in antigen uptake by macrophages and dendritic cells using cell lines and genetically modified mouse lines.

Autoimmunity and molecular immunology

Failure of immunological self-tolerance often leads to the development of autoimmune diseases, which afflict up to 5% of the population. The underlying causes of autoimmune diseases are still unknown, although infections are strong candidates for initiating autoimmunity by promoting the maturation of dendritic cells (DCs) that present tissue self-antigens. This project will analyse the relationship between inflammatory stimuli, the maturation status of DCs that present gastric self-epitopes and the development of organ-specific autoimmunity.

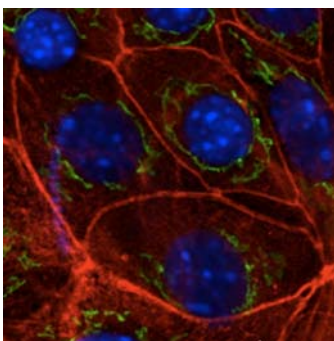


Figure 2. Gastric cells express an autoantigen which is presented in the local draining lymph nodes by migratory dendritic cell

We have established an experimental autoimmune disease of the stomach (autoimmune gastritis) as a powerful model of organ-specific autoimmunity and have identified the gastric autoantigens that are recognized by the self-reactive effector T cells. CD4⁺ T cells that mediate the disease recognize the highly abundant gastric H/K ATPase heterodimer. Immune tolerance to these gastric self-antigens occurs primarily in the periphery. Most importantly, we have also identified a subpopulation of migratory DCs in the draining lymph node of the stomach that presents the endogenous gastric H/K ATPase antigen. Hence, we are now able to compare the status of DCs which present the gastric antigen, from normal mice and mice with autoimmune gastritis, as a basis for understanding the shift from tolerance to autoimmunity. This project will involve the isolation of the migratory population of DCs by standard methods in the lab, analysis of TLR expression of these migratory DCs and assessment of the impact of different TLR ligands on the maturation and functional status of these DCs by analysis of cytokine profiles and activation of gastric-specific effector T cell responses.

Recent publications

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